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Sequence Analysis of the Polysaccharides from *Salmonella newport* and *Salmonella kentucky*

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Structural studies on the lipopolysaccharides (LPS) from *Salmonella newport* (6,8) and *S. kentucky* (8 20) belonging to serogroups C_2 and C_3, respectively, have been reported in previous papers.[1,2] The studies on the *S. newport* LPS were more extensive, and some features in the structure proposed for the O-specific side chains of the *S. kentucky* LPS were not proven but assumed to be the same as in the closely related *S. newport* LPS. Further, the assignment of α-configuration to the D-galactopyranose residue in the *S. newport* LPS seemed to be questionable (Ref. 2, added in proof). Complementary studies on the structure of the O-specific side chains of these LPS have therefore been undertaken and are reported in the present communication.

Angyal and James [3] observed that fully acetylated glycopyranosides with an axially orientated aglycone in the most stable chair form are oxidised only slowly by chromium trioxide in acetic acid. The corresponding anomers, however, with an equatorially orientated aglycone, are readily oxidised, yielding acetylated 5-ketoaldonic esters. We have recently showed that this reaction can be used to determine the anomeric nature of sugar residues in oligosaccharides [4] and polysaccharides.[4,5] It seemed possible that methylation analysis of an acetylated and oxidised polysaccharide may give information on the mutual order of sugar residues in the polysaccharide. This has now been investigated on the *S. newport* and *S. kentucky* LPS.

The fully acetylated LPS were oxidised with chromium trioxide in acetic acid, recovered by gel filtration and subjected to sugar analysis (Table 1). In the sugar analysis of the original LPS,[1] destruction of abequose (3,6-di-deoxy-D-*xylo*-hexose) was minimised by performing the hydrolysis in two steps, with isolation of abequose after the first, mild step. No such precautions were taken in the analysis of the oxidised samples. Further, α-abequosides are oxidised at a faster rate than other α-glycosides, probably because of the lower dif-

Table 1. Sugar analysis of original and acetylated-oxidised LPS. (It is assumed that the D-mannose contents were the same before and after oxidation.)

Sugar	*S. newport*		*S. kentucky*	
	Rel. molar proportion before oxidation	Rel. molar proportion after oxidation	Rel. molar proportion before oxidation	Rel. molar proportion after oxidation
Abequose	15	10	16	5
L-Rhamnose	15	3	17	2
D-Mannose	30	30	36	36
D-Galactose	18	7	20	7
D-Glucose	12	11	8	4

ference in energy between the two possible chair forms. The lower percentage of abequose in the oxidised samples is therefore not in conflict with the assignment of α-configuration to the abequopyranose residue.

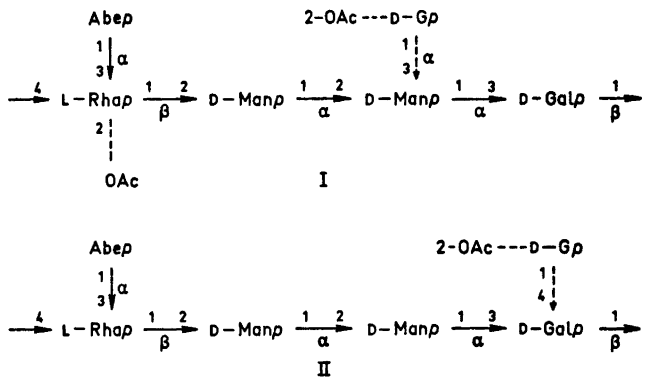
Most of the L-rhamnose residues were oxidised, demonstrating that they are β-linked in the LPS. From the change in optical rotation *versus* time on acid hydrolysis of the LPS it was previously assumed that they were α-linked. Methyl α-L-rhamnoside triacetate is resistant but the corresponding β-L-rhamnoside is readily oxidised by chromium trioxide in acetic acid.[6] As the L-rhamnose residues in the *S. typhimurium* LPS are oxidised [4] but those in the *S. typhi* and *S. strasbourg* LPS are resistant,[5] the former are consequently β-linked and the latter α-linked, in agreement with previous assignments. The fact that abequose is linked to L-rhamnose in the *S. newport* and *S. kentucky* LPS does not weaken the evidence, firstly because considerably more abequose than L-rhamnose resisted the oxidation, secondly because abequosides are not hydrolysed but oxidised to esters during the experimental conditions.[4]

The considerable decrease in the percentage of D-galactose strongly indicates that the D-galactopyranose residues are β-linked. This is in conflict with previous evidence, based upon the NMR of an oligosaccharide obtained by partial acid hydrolysis of the LPS. For polysaccharides, known to contain α-D-galactopyranose residues, the D-galactose content was wirtually unaffected by oxidation.[4] It is therefore assumed that the present evidence is reliable and that the previous evidence was due to a misinterpretation.

In the *S. newport* LPS most of the D-glucose comes from the O-specific side chains. The earlier assignment of α-configuration to these residues is confirmed by the present results, as the D-glucose content was virtually unaffected by the oxidation. (In the original sugar analysis of the *S. newport* LPS the value for D-glucose was 18 %.[1] This value was probably too high, as indicated by the methylation analysis, and the sugar analysis was therefore repeated.)

The decrease in D-glucose content for the *S. kentucky* LPS, from 8 to 4 %, falls within the experimental errors. Although it seems probable that the D-glucose residues in the O-specific side chains of this LPS are also α-linked, this is not well established.

The previously proposed structures for the repeating units of the *S. newport* (I) and *S. kentucky* (II) LPS should therefore be modified as indicated below.



Substituents, connected by a dotted line, occur only in part of the repeating units. As mentioned above, the mutual order of the sugar residues in I was demonstrated by partial acid hydrolysis and identification of oligosaccharides. The mutual order of the second (right) D-mannose residue and the D-galactose residue in II was not determined but assumed to be the same as in I.

Further information was obtained by comparison of the results from methylation analyses of orginal and oxidised LPS (Table 2). As β-linked sugar residues are oxidised to 5-ketoaldonic ester residues, the ester linkages are cleaved during the Hakomori methylation [7] and the thus released hydroxyl groups methylated, new methylated sugars are obtained which give information on sequences. Thus, the 2,3,4,6-tetra-*O*-methyl-D-mannose formed from the oxidised *S. newport* LPS demonstrates that one of the β-linked sugars, known to be L-rhamnose, is linked to the mannose residue that does not carry a D-glucose residue in the 3-position. As considerable amounts of 3,4,6-tri-*O*-methyl-D-mannose and 4,6-di-*O*-methyl-D-mannose are obtained, the α-linked mannose residues must be adjacent and the terminal D-glucose residue α-linked, which confirms previous results.[1,2] The formation of comparable amounts of 2,3,4,6-tetra-*O*-methyl-D-mannose and 3,4,6,-tri-*O*-methyl-D-

Table 2. Methylation analysis of orginal and oxidised LPS. (It is assumed that the total amount of D-mannose ethers should be the same after oxidation. Only the major components of the methylation analysis of the original LPS are given.)

Sugar	*S. newport* Rel. molar proportion		*S. kentucky* Rel. molar proportion	
	unox.	ox.	unox.	ox.
2,3,4,6-Tetra-*O*-methyl-D-glucose	10.3	6.4	48.0	3.0
2,3,4,6-Tetra-*O*-methyl-D-mannose	–	12.8	–	17.7
2-*O*-Methyl-L-rhamnose	14.1	1.3	16.0	–
3,4,6-Tri-*O*-methyl-D-mannose	21.3	11.5	34.4	16.9
2,4,6-Tri-*O*-methyl-D-galactose	13.7	5.1	13.3	3.5
4,6-Di-*O*-methyl-D-mannose	10.0	6.9	–	–

mannose from the oxidised *S. kentucky* LPS demonstrates that the D-mannose residues in the repeating unit are adjacent, as was previously assumed in analogy with the *S. newport* LPS.[1] It is difficult to avoid contaminations during the acetylation and oxidation of the LPS. Minor components of non-carbohydrate nature complicate GLC of the methylated sugars, as their alditol acetates, and make the quantitative evaluation less accurate, and therefore minor components have been omitted for Table 2.

The separation of the derivatives of 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-mannose was incomplete and also for that reason the values in Table 2 are less accurate.

EXPERIMENTAL

General methods. Concentrations were carried out at diminished pressure at bath temperatures not exceeding 40°. GLC was carried out on columns (200 × 0.3 cm) containing 3 % (w/v) of ECNSS-M or 3 % OV-225 on Chromosorb G (80 – 100 mesh) at 195° (alditol acetates) or at 170° (partially methylated alditol acetates). A Perkin-Elmer 900 Gas Chromatograph with flame-ionisation detector was used. For mass spectrometry the mixture of alditol acetates dissolved in chloroform, was injected into an OV-225 SCOT column, mounted in a Perkin-Elmer 270 gas chromatograph-mass spectrometer. The mass spectra were recorded at a manifold temperature of 200°, ionisation potential of 60 eV, ionisation current of 80 μA and an ion source temperature of 120°.

Acetylation of lipopolysaccharides. The LPS [1] (50 mg) was dissolved in formamide (10 ml) and acetic anhydride-pyridine (1:1, 10 ml) was added to the solution, which was kept overnight at room temperature. It was then added to the top of a Sephadex LH20 column (60 × 4 cm) and the column was irrigated with acetone. The acetylated polysaccharide (60 mg), detected by its optical rotation, was eluted with the void volume free from the reagents.

Oxidation of acetylated lipopolysaccharides. Finely powdered chromium trioxide (75 mg) was added to a solution of acetylated LPS (25 mg) in acetic acid (0.75 ml). The suspension was agitated in an ultrasonic bath at 50° for 1 h. The modified LPS (20 mg) was isolated from the filtered solution by chromatography on a Sephadex LH20 column (40 × 2 cm) as described above.

Sugar analyses. The acetylated LPS (5 mg) was heated with 90 % aqueous formic acid at 100° for 1 h. After concentration to dryness the residue was hydrolysed for 16 h with 0.25 M aqueous sulphuric acid. The hydrolysate was then reduced with sodium borohydride and acetylated. The alditol acetates were analysed by GLC[8] – MS.[9]

Methylation analyses. The modified LPS (10 mg) was methylated by the Hakomori procedure [7] as previously described.[10] The reaction mixture was partitioned between chloroform (10 ml) and water (10 ml), and the chloroform phase washed with water of pH3 (4 × 10 ml). The chloroform phase was concentrated to dryness and the methylated product hydrolysed and analysed by GLC – MS as previously described.[10]

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